PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : B01L 7/00, C12Q 1/68, C12M 1/38, B01J 19/00 — A1

(11) International Publication Number: WO 98/09728

(43) International Publication Date: 12 March 1998 (12.03.98)

(21) International Application Number: PCT/GB97/02421

(22) International Filing Date: 8 September 1997 (08.09.97)

(30) Priority Data:
9618595.4 6 September 1996 (06.09.96) GB

(71) Applicant *(for all designated States except US)*: CENTRAL RESEARCH LABORATORIES LIMITED [GB/GB]; Dawley Road, Hayes, Middlesex UB3 1HH (GB).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: CORLESS, Anthony, Robert [GB/GB]; Schillings, Pound Farm Lane, Ash GU12 6EG (GB).

(74) Agent: LEAMAN, Keith; QED Patents Limited, Dawley Road, Hayes, Middlesex UB3 1HH (GB).

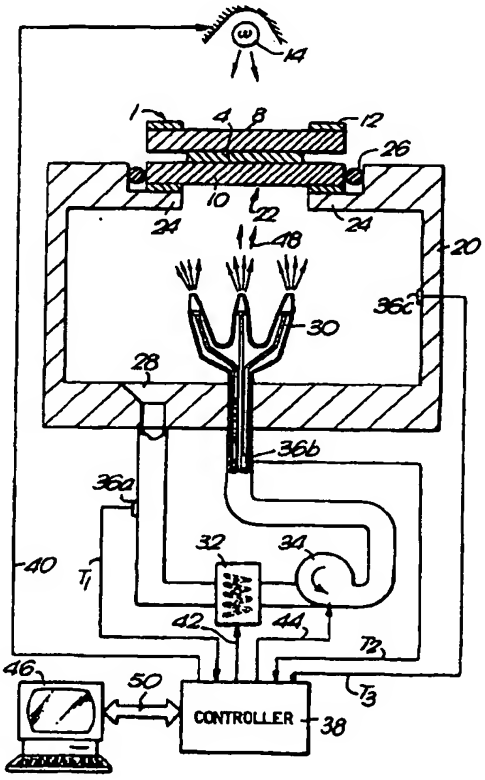
(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: APPARATUS FOR, AND METHOD OF, THERMALLY CYCLING A SAMPLE

(57) Abstract

A thermal cycling apparatus and method particularly suitable for Polymerase Chain Reactions (PCR'S) comprises a disposable unit (2) for receiving a sample (4), a heater (14) and a cooler (32). Heating and cooling occur very quickly and are controllable to a high degree of precision, thereby enhancing the rate of the PCR. In a preferred embodiment a housing (20) is provided in which heating is achieved by an infra-red heater (14) and cooling is by way of a jet (48) of liquid coolant. In an alternative embodiment heating and cooling may be achieved using liquids which are heated, cooled and delivered via different fluid circuits. The invention overcomes problems associated with prior art systems by providing a cheaper apparatus which has less thermal inertia than existing systems and is therefore capable of performing thermal cycling of a sample (4) faster than has previously been possible. In addition, because a disposable sample unit (2) is used, the unit (2) may easily be removed and replaced thereby reducing the risk of cross contamination and increasing the throughput rate of samples.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania
AM	Armenia
AT	Austria
AU	Australia
AZ	Azerbaijan
BA	Bosnia and Herzegovina
BB	Barbados
BE	Belgium
BF	Burkina Faso
BG	Bulgaria
BJ	Benin
BR	Brazil
BY	Belarus
CA	Canada
CF	Central African Republic
CG	Congo
CH	Switzerland
CI	Côte d'Ivoire
CM	Cameroon
CN	China
CU	Cuba
CZ	Czech Republic
DE	Germany
DK	Denmark
EE	Estonia
ES	Spain
FI	Finland
FR	France
GA	Gabon
GB	United Kingdom
GE	Georgia
GH	Ghana
GN	Guinea
GR	Greece
HU	Hungary
IE	Ireland
IL	Israel
IS	Iceland
IT	Italy
JP	Japan
KE	Kenya
KG	Kyrgyzstan
KP	Democratic People's Republic of Korea
KR	Republic of Korea
KZ	Kazakstan
LC	Saint Lucia
LI	Liechtenstein
LK	Sri Lanka
LR	Liberia
LS	Lesotho
LT	Lithuania
LU	Luxembourg
LV	Latvia
MC	Monaco
MD	Republic of Moldova
MG	Madagascar
MK	The former Yugoslav Republic of Macedonia
ML	Mali
MN	Mongolia
MR	Mauritania
MW	Malawi
MX	Mexico
NE	Niger
NL	Netherlands
NO	Norway
NZ	New Zealand
PL	Poland
PT	Portugal
RO	Romania
RU	Russian Federation
SD	Sudan
SE	Sweden
SG	Singapore
SI	Slovenia
SK	Slovakia
SN	Senegal
SZ	Swaziland
TD	Chad
TG	Togo
TJ	Tajikistan
TM	Turkmenistan
TR	Turkey
TT	Trinidad and Tobago
UA	Ukraine
UG	Uganda
US	United States of America
UZ	Uzbekistan
VN	Viet Nam
YU	Yugoslavia
ZW	Zimbabwe

APPARATUS FOR, AND METHOD OF, THERMALLY CYCLING A SAMPLE

The present invention relates to an apparatus for, and method of, thermally cycling a sample. The invention is suitable for, but not limited to, carrying out biochemical processes, particularly, but not exclusively, polymerase chain reactions (PCRs).

PCR is a well known technique for DNA amplification and is described in, for example United States Patent US-A-4,683,202. Generally PCR involves multiple thermal cycling of DNA steps for increasing the DNA yield.

In biochemical processes, particularly PCR, it is desirable to change the temperature of a solution of DNA rapidly with a high degree of precision. Such temperature changes may be from a cooler to a hotter temperature or *vice versa*.

It is believed that efficiency and specificity of amplification of DNA in Polymerase Chain Reactions are increased if rapid, accurate thermal transitions are achieved. This is believed to be the case not only when heating from a lower to a higher temperature, but also when cooling from a higher to a lower one. Rapid cooling of a sample permits rapid binding to complementary DNA chains, such as templates or probes, thereby leading to rapid and accurate amplification of DNA.

United Kingdom Patent GB-A-2233476 (Dean and Evans) discloses apparatus for controlling the temperature of a reaction so that it follows a required temperature/time profile. A multi-well support having a plurality of reaction sites is heated by a quartz halogen lamp and cooled by a fan. The multi-well support is metallic and heats/cools reaction sites by thermal conduction. A microprocessor and temperature sensors control thermal cycling. As the radiation heats, and the fan cools, the multi-well support, (which in turn heats, and cools, the reaction sites) the time taken is relatively longer and the energy imparted to the system (reaction sites, reagent and multi-well support) during the heating phase, which must then be removed during the cooling phase, is significantly greater than the energy required merely to heat the reagents the time taken for each thermal cycle is increased to an undesirable extent.

Also it is generally accepted that, from an engineering point of view, it is easier to heat objects rather than to cool them. United States Patent US 4,865,986 (Coy

Corporation) describes and claims apparatus for selectively heating and cooling upright containers. The apparatus is complex and expensive. In a preferred embodiment heating is performed using electrical resistance heaters. Because the apparatus is bulky there is relatively large thermal inertia. Also unless extremely high electrical currents are used, resistance heaters can take a relatively long time, typically one or two minutes, to heat an object. This means that the apparatus is not suitable for rapid thermal transitions from one temperature to another.

Published International Patent Application WO-A1-9515671 (Inceltec) describes an apparatus which, to some extent, solves some of the aforementioned problems. The apparatus uses a microwave heater which, when switched on and off, subjects a sample to successive heating and cooling cycles. However, the apparatus is complex and requires elaborate shielding in order to prevent microwaves leaking. Also because of the large thermal bulk of the apparatus it is believed that rapid thermal transitions, in particular rapid cooling, is not readily achievable at the rates at which PCR experiments are expected to operate most efficiently.

Thus in order to achieve good results in PCR experiments two criteria are desirable: firstly temperature transitions must be achieved as quickly and as evenly as possible throughout the bulk of a sample; and secondly when a desired temperature is reached this temperature is maintained without any overshoot or dipping. The first criteria implies that the apparatus should have a small heat capacity and good thermal conductivity. The second criteria imposes design restraints on the thermal impedance of the apparatus as well as any control equipment used to monitor sample temperature and to drive any heater(s) and/or cooler(s).

Furthermore in many instances it is desirable that the reagents of a sample and the reaction products are contained within a disposable unit, which should be as cheap and as simple to manufacture as possible. Thus the heating and cooling means should not form part of the disposable unit. However, a heating and/or cooling means external to the disposable unit normally introduces extra thermal impedance, which implies that less precise and less rapid thermal cycling is achievable.

An aim of the present invention is to provide an apparatus for, and method of, thermally cycling a sample sufficiently rapidly and with sufficient accuracy so as to be suitable for biochemical reactions and in particular polymerase chain reactions (PCRs) in order to increase the efficiency of the reaction.

Another aim of the invention is to provide an apparatus for, and a method of, thermally cycling a sample which overcomes the aforementioned problems associated with prior art systems.

A further aim of the present invention is to provide an apparatus for, and a method of, thermally cycling a sample sufficiently rapidly and with sufficient accuracy so as to be suitable for biochemical reactions and in particular polymerase chain reactions (PCRs) at very high speed, such that the time taken to complete the PCR process is substantially reduced compared to that achieved by the prior art.

According to the present invention there is provided a method of thermally cycling a sample, the method comprising:

i) providing a sample in a disposable unit and arranging the sample in the unit so that the sample is in thermal contact with a cooling means;

ii) providing a source of infra-red radiation so that the radiation is absorbed by the sample and not absorbed to any extent by either the disposable unit or the heat sink means, thereby in use permitting a rapid thermal transition from a lower sample temperature to higher sample temperature to be achieved;

iii) providing a coolant for cooling the disposable unit so that the temperature of the sample is reduced rapidly; and

iv) repeating the aforementioned heating and cooling until a desired state of the sample is reached

Preferably the reaction is a PCR. The PCR may be used to amplify DNA.

In considering the achievement of these aims, useful guidance on likely performance may be gained by consideration of the thermal capacity of the sample and the thermal resistance of the path through which the heat must travel. The thermal path includes a path through the sample and through all elements to the ultimate cooling means. A "time constant" for the system is given by the product of the heat capacity and thermal resistance. Minimisation of this time constant implies an overall improvement to the performance. According to another aspect of the present invention there is provided apparatus for thermally cycling a sample, in use the sample being contained in a disposable sample unit, the apparatus comprising:

a housing for receiving the disposable sample unit so that it is in thermal contact with a heat sink, or otherwise directly accessible to a cooling means;

infra-red heating means for heating the sample;

cooling means for cooling the sample, and

means for synchronising the heating and cooling means so that rapid thermal cycling of the sample is achieved according to a desired thermal cycling profile.

Preferably the disposable unit is in the form of a planar structure, that is an object which has a thickness small compared to its major axis in the plane, such as a plate or disc, and is such that either both major faces are thermally conducting (to allow double sided heating and cooling) or such that one major face is thermally conducting and the opposite major face is essentially thermally insulating. This allows single sided heating and cooling substantially free of effects due to conduction of heat through the one major face. Manufacturing the disposable unit in this way allows a high surface area to volume ratio allowing heat to be transported into and out of the disposable unit rapidly. For the most part, the temperature inside the disposable unit, that is the temperature of the sample, will equilibrate by thermal conduction. This sets a minimum possible time constant for the apparatus. Preferably the thickness of the sample, that is the separation between the major faces of the disposable unit, is less than 2.5×10^{-3} m, more preferably it is less than 1×10^{-3} m, yet more preferably the thickness of the sample is approximately 100 10^{-6}m, with a yet thinner layer being preferable until a limiting thinness is reached at which surface effects constrain the biochemical reactions. The present state of knowledge prevents absolute specification of this lowest limit.

Preferably cooling of the disposable sample unit is achieved by directing a coolant onto the disposable unit or heat sink(s) thermally connected thereto and by thermal conduction and/or forced convection via the heat sinks. Fins, grooves or other means may be applied to the surface(s) of the disposable unit and/or heat sink(s) so as to increase their surface area.

Preferably the heat sink(s) and any mechanically robust elements of the disposable unit which are in the thermal path are manufactured from a material which has a high transmissivity to infra red radiation employed and a high thermal conductivity. More preferably these elements are manufactured from silicon or from diamond. Silicon is presently the most preferred material since its cost is substantially less than that of diamond.

Preferably the wavelength of infra-red radiation of interest is between 1.2×10^{-6} m and 25×10^{-6} m. More preferably the wavelength of radiation is between 1.4×10^{-6} m and 15×10^{-6} m.

A preferred background source of radiation (which may optionally be used to maintain the sample temperature allowing a continuous balance between heating and cooling) is black body radiation filtered, for example using a silicon sheet to remove short wavelengths which may cause undesirable heating of the assembly. The source temperature is chosen so as to maximise the radiation available at wavelengths above 1.4×10^{-6} m where water has its most rapid absorption. Most preferably the bulk of radiated energy is in the region of the strong absorption peak in the region of 3×10^{-6}m wavelength. Such a source is an ideal source of a background radiation, but is less suited to precise, rapid modulation required for rapid temperature change. It is understood and accepted that varying electrical power supplied to a heated source, mechanical shutters, and other arrangements, may be contrived to effect such modulation.

A presently preferred source of infra red power which is capable of rapid modulation is a carbon dioxide gas laser. Such lasers are readily available in power levels from a few Watts to a few kilo Watts. The beam power may be readily, and rapidly, modulated and the beam may be readily directed. Such a source may be employed in addition to the black body source, as discussed above, or it may be used as the sole infra red source, having its power adjusted as required to raise the temperature, maintain the temperature or allow cooling.

Advantageously heating and cooling are performed as rapidly as possible so as to reduce the overall thermal cycle time. Preferably temperature transitions from around 25°C to within a range of 90–98°C are achieved in less than one second. Most preferably the aforesaid temperature transitions are achieved in less than 0.1 second. Precision of temperature measurement is preferable to within $\pm$ 0.5°C.

Thus in accordance with the invention, the sample is directly heated and once heating is stopped, or reduced in intensity, the sample is rapidly cooled at a controlled rate by being in contact with a heat sink which is maintained at a low temperature, or with a cooling means. It is thus possible to thermally cycle the sample more rapidly than has been previously possible.

In another preferred embodiment, the heat sink may be formed integrally with the disposable unit. In a further embodiment which is presently preferred a low-cost

disposable unit is removable from a housing. The disposable unit comprises a plastics envelope, and the heat sink means comprises two spaced apart sheets or slabs of silicon, which are moved to grip the faces of the plastics envelope. The arrangement thus formed is hereinafter termed a reaction cell. The advantage with a disposable unit is that it contains all sample materials in a sealed environment and may be removed after an experiment or reaction and easily and readily replaced without having to clean or modify any ancillary heating, cooling or control equipment. This enables higher throughput with less risk of cross-contamination. In this embodiment the plastics material is preferably maintained as thin as is practically possible having regard to its mechanical function; namely it must be sufficiently strong to support its own weight.

In a yet further embodiment which is presently most preferred as a means of minimising the cycle time, the disposable unit comprises a silicon base, optionally coated with a thin layer of material which renders it biologically compatible with the PCR process, and a plastics envelope forming the sidewalls and top of a reaction cell. The sidewalls and top are essentially thermally insulating with all salient heat transfer being through the thin biocompatible layer and the silicon base. Preferably the thin layer is biocompatible and comprises one of the native oxides, oxynitrides or nitrides of silicon and/or a plastic layer, applied to the silicon, for example, by means of spin casting followed by drying or curing as required. The techniques of spin casting polymer layers, such as polyimide, are well-known to artisans of ordinary skill in the microelectronics industry. Preferably the thin biocompatible layer will have a thickness in the range 0.1–10 microns, more preferably in the range 0.5 to 3 microns.

According to a different aspect of the invention there is provided apparatus for thermally cycling a sample, in use the sample being contained in a disposable sample unit, the apparatus comprising:

a housing for receiving the disposable sample unit so that it is in thermal contact with a heat sink or directly with a fluid heat transfer means;

heating means comprising a fluid heater and a pump for pumping the fluid so that the fluid heats the sample;

cooling means for cooling the sample, and

means for synchronising the heating and cooling means so that rapid thermal cycling of the sample is achieved according to a desired thermal cycling profile.

Preferably however, infra-red radiation is employed to heat the sample, as the sample usually contains a high percentage of water, which strongly absorbs infra-red radiation. Plastic, or polymeric, material may be selected such that it is relatively non-absorbant of infra red radiation, at least at specific wavelengths of interest and is therefore appropriate for forming the disposable unit. The radiation may optionally be filtered, at, or close to, its source, by passing it through a sheet of said plastic or polymeric material thereby removing, or reducing in intensity, such wavelengths as are absorbed while passing the remainder of the radiation. The heat sink(s) are preferably formed from silicon, because silicon is transparent to infra-red radiation and has good thermal conductivity, and is a relatively low-cost and readily available material.

The wavelength of the infra-red radiation may be selected so that most of the radiation passes through the sample, with less than 10%, being absorbed by the sample. This provides a means of uniformly heating the sample. Alternatively, the wavelength of radiation is selected so that the radiation is almost completely absorbed by the sample in a thin layer, with the remainder of the sample being heated principally by conduction from that layer. In the case of a sample which is continuously cooled it is preferable to supply heat by radiation which is absorbed in the thin layer closest to the cooling means sufficient to compensate for the loss of heat to the cooling means, except at such times as when the temperature is reduced. The temperature is raised by either an increase in the radiation level absorbed in said thin layer or by radiation of a wavelength which is absorbed more uniformly throughout the sample thickness. Provision may be made to heat and cool the sample from two sides of the disposable unit so as to provide for more uniform irradiation induced heating and cooling. The irradiation may be achieved by using more than one infra-red source, one radiation source and a combination of mirrors and beam splitters or by way of an alternative heater such as a heated fluid.

In a further modification, a reflecting layer of, for example gold, may be disposed on or adjacent a surface of the sample unit on the side of the sample opposite the incoming direction of radiation.

If the sample is cooled and irradiated through only major face of the unit then preferably the major face opposing said first major face is substantially thermally insulating.

Peltier electrode structures may be coupled to the heat sink(s) in order to induce cooling of the heat sink(s). The Peltier structures may be permanently coupled to the heat

sink(s); or, in a further modification, where the heat sink(s) are formed integrally with the disposable sample unit, they may be removably attached to the heat sink(s). In an alternative embodiment, for example in which cooling is achieved by fluid, Peltier coolers are omitted and in this case the heat sink(s) may also be omitted.

The reaction cell, in which the sample unit is supported, is preferably dimensioned and arranged so as to be received by a housing. The housing, may be in the form of an airtight vessel with an aperture for receiving the disposable unit. An O-ring seal may be arranged to seal the periphery of the aperture between the housing and the sample reaction cell.

Preferably environmental sensors are provided within the housing which measure pressure and/or temperature and supply signals to a controller.

In a further preferred embodiment the housing includes forced cooling means such as, for example, a pump connected to a nozzle arranged to supply a coolant so that the coolant is directed onto one surface of the disposable unit or heat sink(s) thereby enhancing the rate of removal of heat.

Coolant is drained from the vessel, cooled and returned to the pump. The pump, whose rate may be varied, is arranged to deliver coolant under control of a micro-processor. The micro-processor may have one or more inputs from the environmental sensors and is preferably arranged to control the temperature of the cooler and the heat source(s). In this arrangement, as in the previous, the micro-processor can be "taught" how to control the temperature, by way of neural networks and/or knowledge of a response function of the housing.

Preferably a liquid coolant is selected whose infra-red absorption is very low, when compared with that of the sample and most preferably negligible, in the spectral range of any irradiation used. Advantageously, the liquid coolant may be selected to have a boiling point at or around a temperature of interest. Liquids such as alcohols, paraffins or perfluorpolyethers are envisaged as being appropriate, especially as some of these can be tailored to have boiling points at or around temperatures of interest. By tailoring the coolant, so that there is a phase change at or around the temperature of interest, it is possible to remove large amounts of heat extremely quickly as a result of utilising heat absorbed at phase changes such as the latent heat of vaporisation . The use of a coolant which vaporises at, or close to, a desired control temperature is a presently

preferred means of ensuring that the heat transfer surface remains at a constant relatively low temperature by vaporising said coolant.

A further means of maintaining the heat transfer surface at a constant temperature is to arrange a jet, or jets, of gas periodically to blow the spent coolant from the said surface. This prevents insulating layers of coolant developing.

A yet further means of maintaining a defined level of coverage of the heat transfer surface is to arrange that the reaction cell rotates within the housing such that centrifugal forces cause the any coolant adhering to the surface to be expelled from the surface.

A suitable control algorithm and/or neural network may be taught to recognise changes in physical characteristics of a sample. As the sample is amplified its specific heat capacity, conductivity and consequently variations in heating and cooling rates are required and optical (uv, visible and infra-red) absorption or fluorescence characteristics may vary. Information derived from the aforesaid variations may be used to monitor the progress of the reaction and/or to control the rate of reaction.

Variations in the control of the rate of reaction can be achieved either analytically, as mentioned below, or by checking data with stored data on a look-up table. Similarly status checks may be made by the micro-processor as to how far reactions have progressed and at what stage reactions should be terminated. Such status checks may also be performed automatically. One way this may be achieved remotely is by shining a laser into the sample and determining how it is reflected or absorbed by using a photocell. A response function for the housing may be convoluted with a response function of the disposable unit so as to obtain a control function for the heating source(s).

By judicious choice of coolant, thermal conductivity, specific heat capacity of the disposable unit, coolant mass flow rates, and wavelength and duration of infra-red radiation, it is possible to effect extremely rapid and precise thermal transitions of the sample in the disposable unit.

In a further alternative embodiment both heating and cooling are achieved using a forced fluid convection system. The system comprises a housing for receiving a disposable unit; at least two fluid delivery circuits capable of supplying fluids at different temperatures, so that one of the circuits supplies fluid hotter than the other; and control means for synchronising delivery of the fluids by way of at least one valve, so that in

use, the disposable unit is heated by supplying the hotter fluid and cooled by supply of the relatively cooler fluid.

The system may include infra-red heaters and where appropriate features of other embodiments already described above.

In a yet further embodiment both heating and cooling are achieved using a forced fluid convection system in which a single working fluid is taken from a cooled reservoir and is heated to a precisely defined temperature as it flows to an outlet nozzle. Preferably such heating means take the form of a compact heater with low liquid capacity capable of rapid temperature changes, such as a resistively heated sintered, or otherwise fabricated, porous element.

Preferred embodiments of the invention will now be described, by way of example only, and with reference to the Figures in which:

Figure 1 is a sectional view of a sample reaction cell comprising a disposable sample unit mounted between two heat sinks;

Figures 2 to 4 show sections through alternative embodiments of the invention;

Figure 5 is a graph of an example of an idealised thermal cycle applied to a sample in a PCR.

Figure 6 shows a section through a further embodiment of the invention; and

Figure 7 shows is a sectional view through an alternative embodiment of a sample reaction cell.

Referring now to Figure 1, a sample reaction cell 1 comprises a disposable planar disc shaped unit 2 and two heat sinks 8 and 10. Unit 2 is formed from synthetic plastics, such as polypropylene or polycarbonate, and in use receives a liquid sample 4. The sample 4 is mainly water in composition and contains DNA and other substances. Unit 2 is tightly sandwiched between two heat sinks 8 and 10 formed from silicon sheets. The sandwiched arrangement thus formed is hereinafter referred to as a reaction cell 1. It is noted that the reaction cell may contain a plurality of samples in an arrangement of wells.

Unit 2 is preferably not more than 2600 microns thick, and its walls are preferably not more than 50 microns. More preferably the walls are as thin as is compatible with mechanical handling. The thickness of the sample 4 is thus reduced to a minimum compatible with the basic biochemical processes. These features are beneficial

to the reduction of the thermal time constant of the reaction cell. The area of the cell is not as important to the thermal performance as is its thickness, assuming that the extent of the cell 1 in the plane is significantly greater than its thickness, and that its area is uniformly coupled to heating and cooling means. Typically, the wall thickness of the disposable unit is in the range 5–25 microns and the sample thickness is in the range 100–1000 microns. The diameter of the unit is in the order of 10 mm. For typical plastics materials this range yields a "thermal time constant" for the reaction cell 1 which is in the range of approximately 0.02–1.0 seconds. Unit 2 provides a disposable container for receiving the sample 4 and facilitates low cost and rapid fabrication.

Heat sinks 8 and 10 are typically, 5×10^{-3} m thick and are positioned above and below the unit 2 and in good thermal contact therewith, so as to ensure good heat transfer to and from disposable unit 2. Disposed around the edges of each heat sink 8 and 10 are Peltier devices 12. These devices are optional. One or more infra-red radiation sources 14 are positioned above the heat sinks 8 and 10 and provide infra-red radiation to irradiate the reaction cell 1 whilst not impinging on the Peltier devices 12 if present.

To achieve rapid cooling, heat sink 8 is in excellent thermal contact with the unit 2 and thereby sample 4 is always maintained at a temperature at or below the lowest temperature in the thermal cycle. Plastics materials generally have a low thermal conductivity, allowing a temperature gradient to be established through the thickness of the wall of unit 2 with little heat transfer. However, as the wall is very thin this effect is negligible. To heat the sample 4, a flux of infra-red radiation is used, and by judicious choice of the range of wavelengths, it can be arranged that very little radiation is absorbed by the unit 2, and that useful absorption takes place in the sample 4. The radiation flux is chosen to exceed, balance, or be less than the heat flow through the walls and into the heat sinks 8 and 10. In this way the sample temperature increases, can be maintained constant, or falls in response to the amount of heating.

In order to achieve good heat transfer from the walls of the unit 2, and thereby permit rapid cooling, the material from which the heat sinks 8 and 10 is made should be a good thermal conductor. Also, to permit a flux of infra-red radiation into the unit 2 the material from which it is made should be transparent to such radiation. A material which offers both good thermal conductivity and transparency to infra-red radiation is silicon. Heat is removed from the silicon heat sinks 8 and 10 by, for example, Peltier devices 12 which are arranged to maintain an even and controlled rate of heat removal. By mounting

Peltier devices 12 around the periphery of the silicon heat sinks 8 and 10, a region is available for infra-red illumination of sample 4. The good thermal conductivity of the silicon heat sinks 8 and 10 serves to give lateral heat spreading and a substantially uniform temperature profile throughout the disposable unit 2.

In operation unit 2, containing sample 4, is introduced between the heat sinks 8 and 10. A clamping or vice structure (not shown) is employed to move the heat sinks 8 and 10 from an open position in which the sample unit 2 is positioned between the heat sinks, to a closed position (as shown in Figure 1), in which the sample unit 2 is firmly gripped between the heat sinks 8 and 10; and is therefore in good thermal contact with them.

Infra-red radiation source 14 operates to maintain the sample 4 at a desired initial temperature. When thermal cycling is commenced, the output of radiation source 14 is raised in intensity to heat the sample 4 quickly to a desired temperature for a desired time. The infra-red source 14 may then be reduced in intensity in a desired manner to enable the sample 4 to cool back to its initial or other desired state.

Heat sinks 8 and 10 dissipate heat by way of conduction and natural convection, or serve to conduct the heat to the optional Peltier devices. As mentioned above the rate of dissipation may be enhanced by forming fins in the heat sinks. However, unit 2 or the reaction cell 1 may be incorporated into an apparatus adapted to increase the rate of cooling of the sample 4, by forced convection. Embodiments of this aspect of the invention will now be described with reference to Figures 2 to 4.

Referring now to Figures 2 to 6, in which like parts bear the same reference numerals, there is shown a housing 20 which is in the general form of a right circular cylinder. Housing 20 is formed from glass or synthetic plastics material. Housing 20 has an aperture 22 in its upper surface. A shelf 24 is defined within aperture 22. Shelf 24 is dimensioned and arranged to receive the sample reaction cell 1 (i.e. sample unit 2 and at least one heat sink) or the sample unit 2. In the embodiment shown the sample reaction cell 1 has a DNA sample 4 contained in disposable sample unit 2. An O-ring seal 26 seals a gap between the housing 20 and the edge of reaction cell 1. The O-ring seal therefore ensures the housing 20 is airtight. At the bottom of the housing 20 there is a drain 28. Nozzle 30 projects into the housing 20. The drain 28 is arranged to drain any coolant fluid from within the housing 20 and deliver the coolant to a cooler 32. Cooler 32

is arranged to cool any fluid collected by drain 28 and to deliver the fluid to nozzle 30 via pump 34.

Temperature sensors 36 sense the temperature of the fluid at different positions in the housing 20. Temperature sensor 36a provides an indication of the temperature of the fluid being drained from the housing 20. This temperature is indicated by T_1. Temperature sensor 36b provides an indication of the temperature of the coolant fluid as it enters nozzle 30 and this is indicated by T_2. Temperature sensor 36c senses the temperature within the housing 20 and this is indicated by T_3. Signals indicative of temperatures T_1, T_2 and T_3 are input into a controller 38 and the information is stored in and manipulated by computer 46. Computer 46 communicates with the controller 38 by way of a bi-directional data bus 50.

Control signals 40, 42 and 44 are output from controller 38. Control signal 40 switches infra-red heating source 14 on and off. Control signal 42 controls cooler 32. Control signal 44 controls pump 34.

The apparatus will now briefly be described in its operation and with reference to the graph shown in Figure 5. Sample reaction cell 1 is placed within aperture 22 and O-ring seal 26 is fitted snugly between the cell 1 and wall of housing 20. Information as to the type of polymerase chain reaction (PCR), amount of sample 4, type of sample 4, ambient temperature and thermal cycling profile are input into the computer 46. Software within the computer 46, together with information (which may have been obtained from previous experiments), calculates the desired mass flow rate of coolant. Initially pump 34 pumps coolant slowly. This enables the housing 20, cell 1 and sample 4 to reach an equilibrium temperature and for all sensors 36 to reach steady state. In practise this may only take a few seconds or at most one or two minutes. Once a steady state has been reached rapid thermal cycling may commence. This is achieved by increasing the output pressure of pump 34. The pressure of the coolant delivered via nozzle 30 increases. Jets of fluid 48 impinge on lower surface of heat sink 8 of the reaction cell 1. Jets of coolant (whose temperature is at Tss) maintain the surface temperature of the lower surface of heat sink 8, and therefore the sample 4, at Tss. At a predetermined instant (point A on Figure 5), controller 38 switches on infra-red heating source 14. Source 14 rapidly raises the temperature of the sample DNA to temperature Td. This is shown in Figure 5 as point B. Heating is achieved extremely rapidly for the reasons described above. The

temperature of the sample is typically in the range of 90 - 98°C. At this temperature DNA helices split apart. This phase is known as denaturisation.

During the heating stage of the cycle, coolant may still be pumped onto the heat sink 8 or pumping may be switched off temporarily. The state the experiment has reached is shown by point B on the graph in Figure 5. The temperature is maintained at temperature Td typically for a dwell period. The duration of this dwell period is optimised for the particular PCR reaction, and will generally be minimised.

The intensity of heat source 14 is then reduced, possibly to zero, allowing sample 4 to cool rapidly to temperature Ta, shown as point C on Figure 5. This stage is known as the annealing stage. An optimum temperature for the annealing stage is typically within the range of 45–85°C. This temperature is reached rapidly as a result of the jets 48 of coolant impinging against lower surface of heat exchange 8. Undershoot of the cooling is minimised by both careful consideration of the rate of supply of coolant, (and/or the coolant temperature), and by applying a background radiation level which maintains the sample temperature. During the annealing stage DNA stretches or primers attach to parts of a target sequence.

The next stage of the cycle involves reheating of the sample 4. This period is known as active amplification of DNA and at the onset of this stage the temperature of the apparatus is rapidly increased to Tm. This is shown by point D on Figure 5. During this stage nucleotide extension occurs, thereby forming complementary DNA strands. The optimum temperature range for this stage of the process occurs around 40 - 70°C. Reheating is conveniently arranged by increasing the radiation level to increase and finally maintain the temperature Tm. Conveniently, the coolant may be arranged to have a boiling point at, or close to, the temperature Tm. The complete cycle is then repeated, typically another 20 - 40 times.

It is noted that by modifying the rate of flow of coolant and suitably adjusting a combination of: heater 33, cooler 32 and infra-red source 14 the temperature of the sample 4 can be varied at any time quickly and precisely.

Temperature sensor 36c provides a feedback signal of temperature T_3 which is used to monitor the temperature inside the housing 20. When desired the controller 38 acts to change the temperature of the sample reaction cell 1. Again, this occurs almost instantaneously by virtue of the rapid heat transfer capabilities of the heater 33, cooler 32, pump 34 and infra-red source 14 and also because: the heater 33, cooler 32 and pump

34 are close together; their inter-connecting tubes are short; and they have a low specific heat capacity. Thus a relatively small amount of liquid is held in these components allowing rapid transition of the temperature of the jets 48. In addition, because the operation of heater 33, cooler 32 and pump 34 is controlled by a microprocessor, rapid temperature transits are possible.

When the thermal cycle is complete, an alarm (not shown), which may be an audible alarm, signals to an operator that the reaction is terminated. The reaction cell 1 is then removed and a fresh reaction cell 1 may be quickly and easily inserted in order to undergo the same or a different thermal cycle.

Figure 3 shows a similar arrangement to that shown in Figure 2 in which like parts bear the same reference numerals. Figure 3 shows a housing 20 in which a nozzle housing 30 has three separate nozzles 31 a, b and c. Three separate heating, cooling and pumping circuits are also shown. Each circuit is capable of supplying a fluid at a different temperature. Valves 45 a, b and c are opened and closed in accordance with control signals from controller 38. The operation of the arrangement shown in Figure 3 is similar to that shown in Figure 2, except that in the embodiment shown in Figure 3 there is no infra-red heating source. Heating is performed by way of one or more jets 48 of hot fluid, which is preferably hot water and which emerges through at least one of the nozzles 31. The arrangement may be used in conjunction with an infra-red heating source but this is not necessary because the relatively higher temperature of water required to heat the sample reaction cell 1 is supplied by an electric heater 32a. As one heater 32a is acting to raise the temperature of the liquid to near its boiling point, a one way valve 37 is present in that circuit so as to prevent unwanted back flow into the body of the housing 20. Cooling is achieved in a similar fashion to the embodiment shown in Figure 2, namely by directing a jet of coolant against the heat sink 8. Temperatures of the three fluid channels are separately indicated as Tx, Ty and Tz in Figures 3 and 4.

Figure 4 shows a similar arrangement to that shown in Figure 3. Like parts bear the same reference numerals. However, in this embodiment, the disposable sample unit 2 is in contact with only one heat sink 8. Again an optional infra-red heater may be included to accelerate heating. However, both heating and cooling can be achieved using jets of liquid. It is noted that if heating and cooling employ only jets of liquid then the constraint that the reaction cell be transparent to infra red radiation is no longer applicable. In such an embodiment the reaction cell may be conveniently constructed, as

shown in Figure 7, from a metal base 60 with a plastics envelope 62, 64 defined thereupon. A presently preferred embodiment is an aluminium base 60 with a thin plastics coating 62, (rendering the surface biocompatible), upon which coating 62 is bonded a suitably domed, or pressed, plastics component 64 which defines the volume occupied by sample 4.

Figure 6 shows a similar arrangement to that shown in Figure 2. Like parts bear the same reference numerals. However, in this embodiment, the disposable sample unit 2 is in contact with only one heat sink 8. In this case the infra red heating 14 is shown to enter the disposable sample unit 2 through the heat sink 8, heating the cooled side of the disposable sample unit. This arrangement of directing infra red radiation preferentially to the cooled side of the unit is presently preferred.

Modification may be made to the reaction cell 1 so that it comprises an integral temperature sensor (not shown), such as a moulded thermocouple contact or a thin film thermocouple. Such temperature sensors are accurate to $\pm$ 1.0^{0}C are cheap, stable and inert.

Electric and/or magnetic fields may be arranged to act on the sample so as to manipulate or vary one or more physical characteristics before, during or after the PCR. Also the housing 20 may be incorporated into a centrifuge so that centrifugal and/or vibrational forces are caused to act on the sample 4 so as to enhance mixing or separation.

The stage to which an experiment has progressed may be ascertained by a number of methods. One of these includes launching a low energy laser (not shown) into the sample unit 2 and monitoring the amount of absorption or reflection of the laser by the DNA, by using a photocell or fluorescence detection (not shown). Any of the embodiments described above may include this feature.

Other variations to the aforementioned embodiments may be made without departing from the scope of the invention. For example, and without limitation, the pressure and/or temperature of the interior of the housing 20 may be varied so as to modify the boiling points of liquid jet(s) 48 which impinge on the heat sink 8. The interior pressure of the housing 20 may be reduced relatively simply. It will be noted that suitable non-return and one way valves may be required in fluid channels so as to prevent suck back of any fluid into the housing 20. Also a gasket may be required between the lower surface of heat sink 8 and the shelf 24 on which it sits in the housing

20. Pressure within the housing may be raised in order to increase the boiling point of coolants. This may require clamping of the reaction cell 1 so that it remains in an air tight fit within aperture 22 during an experiment and is not urged out by higher internal pressure within the housing 20.

Other variations and features which may be applied to the disposable unit include: the addition of a code by which the unique disposable unit 2 is identified to computer 46 and in particular from which essential control parameters for the reaction may be conveyed; and/or the addition of a semi conductor "chip" which stores information concerning the reaction cycle undergone by the disposable unit; and/or use of capillary action to fill the disposable unit 2; the use of a tight fitting plug permanently to seal the unit 2 following sample loading; and/or the use of a suitably viscous thermally, or otherwise, cured material to seal a capillary opening following sample loading; and/or the use of a tape seal to seal an aperture (in the unit) used for sample loading; and/or the supply of disposable units predosed with reagents, (particular PCR reagents) in a dried form.

The invention has been described by way of embodiments only and it will be appreciated that variation to the embodiments may be made without departing from the scope of the invention.

CLAIMS

1. Apparatus for thermally cycling a sample (4), in use the sample (4) being contained in a disposable sample unit (2), the apparatus comprising:

a housing (20) for receiving the disposable sample unit (2) so that it is in thermal contact with a heat sink (8, 10);

infra-red heating means (14) for heating the sample (4);

cooling means (32) for cooling the sample (4), and

control means (38) for synchronising the heating (14) and cooling (32) means so that rapid thermal cycling of the sample (4) is achieved according to a desired thermal cycling profile.

2. Apparatus for thermally cycling a sample (4), in use the sample (4) being contained in a disposable sample unit (2), the apparatus comprising:

a housing (20) for receiving the disposable sample unit (2) so that it is in thermal contact with a heat sink (8, 10);

heating means comprising a fluid heater (32a) and a pump (34a) for pumping the fluid so that the fluid heats the sample (4);

cooling means (32c) for cooling the sample (4), and

control means (38) for synchronising the heating (32a) and cooling (32c) means so that rapid thermal cycling of the sample (4) is achieved according to a desired thermal cycling profile.

3. Apparatus according to claim 1 or 2 wherein the sample unit (2) is adapted to be removably inserted into an aperture (22) defined in the housing (20) and means (26) is provided for sealing the aperture (22).

4. Apparatus according to claim 3 wherein sensors (36) are provided in the housing (20).

5. Apparatus according to any preceding claim having a microprocessor (46), for communicating with the control means (38).

6. Apparatus according to claim 5 having means for recognising a change in a physical characteristic of the sample (4), said means being capable of supplying a signal indicative of said change.

7. Apparatus according to claim 6 wherein the means for recognising a change in a physical characteristic of the sample (4) comprises a source of laser radiation directed at the sample (4) and a photocell adapted to receive reflected or transmitted laser radiation.

8. Apparatus according to claim 1 or 2 wherein the cooling means comprises a liquid coolant.

9. Apparatus according to claim 8 wherein the latent heat of vaporisation of the coolant is in the temperature range to which cooling is desired.

10. Apparatus according to any preceding claim wherein the sample unit (2) is formed from synthetic plastics material.

11. Apparatus according to any preceding claim wherein the heat sink means (8, 10) comprises a silicon substrate.

12. Apparatus according to claim 1 and any claim dependant thereon, wherein the wavelength of infra-red radiation lies in the region of 1.2×10^{-6} m and 25×10^{-6} m.

13. Apparatus according to claim 6 or 7 further comprising means (46) for modifying the heating and/or cooling means in response to the change in a physical characteristic of the sample (4).

14. A sample unit (2) comprising a synthetic plastics envelope is adapted for use with the apparatus as claimed in any of claims 1 to 13.

15. A sample unit (2) according to claim 14 wherein the surface of the unit (2) in contact with the sample (4) is an adiabatic layer.

16. A sample unit (2) according to claim 14 or 15 wherein a reflective layer is disposed on or adjacent a surface of the sample unit (2).

17. A method of thermally cycling a sample, the method comprising:

i) providing a sample (4) in a disposable unit (2) and arranging the sample (4) in the unit (2) so that the sample (4) is in thermal contact with a heat sink means (8, 10);

ii) providing a source (14) of infra-red radiation so that the radiation is absorbed by the sample and not absorbed to any extent by either the disposable unit (2) or the heat sink means (8, 10), thereby in use, permitting a rapid thermal transition from a lower sample temperature to higher sample temperature to be achieved;

iii) providing a coolant for cooling the disposable unit (2) so that the temperature of the sample (4) is reduced rapidly; and

iv) repeating the aforementioned heating and cooling until a desired state of the sample (4) is reached.

18. A method according to claim 17 wherein a temperature transition from 25°C to a temperature in a range of 90 - 98°C is achieved in less than one second.

19. A method according to claim 18 wherein said temperature transition is achieved in less than 0.1 second.

20. A method according to any of claims 17 to 19 wherein the method is employed for use in a Polymerase Chain Reaction(PCR).

21. A method according to any of claims 17 to 20 for amplifying a DNA sample.

22. Apparatus according to claim 1 or claim 2 wherein the disposable unit is in the form of a planar structure.

23. Apparatus according to claim 22 wherein the disposable unit is in the form of a planar structure having two major faces both of which are thermally conductive.

24. Apparatus according to claim 22 or claim 23 wherein the thickness of the planar structure is less 2.5×10^{-3} m.

25. Apparatus according to claim 22 or claim 23 wherein the thickness of the planar structure is less than 1×10^{-3} m.

26. Apparatus according to claim 1 wherein the heat sink(s) and a portion of the disposable unit which are in the thermal path, comprise a material having a high transmission of the infra-red radiation employed and also having a high thermal conductivity.

27. Apparatus according to claim 26 wherein the material is selected from the group comprising, silicon and diamond.

28. A method according to claim 17 wherein the wavelength of infra-red radiation of is between 1.2×10^{-6} m and 25×10^{-6} m.

29. A method according to claim 17 wherein the wavelength of infra-red radiation is between 1.4×10^{-6} m and 15×10^{-6} m.

30. Apparatus according to any one of claims 1 to 13 and 22 to 27 wherein there is provided a source for a background radiation level operable to maintain the sample at one or more predetermined temperatures.

31. Apparatus according to claim 30 wherein the source of background radiation is black body radiation filtered to remove short wavelengths so as to prevent undesirable heating of the sample (4).

32. Apparatus according to claim 31 wherein the temperature of the black body radiation is chosen to maximise the radiation available at wavelengths above 1.4×10^{-6} m.

33. Apparatus according to claim 1 wherein the radiation source is a carbon dioxide gas laser.

34. Apparatus according to claim 22 wherein a region adjacent one surface of the sample unit is thermally insulating and a region adjacent the other surface is thermally conductive.

35. Apparatus for thermally cycling a sample substantially as herein before described with reference to the Figures.

36. Method of thermally cycling a sample substantially as herein described with reference to the Figures.

Fig.1.

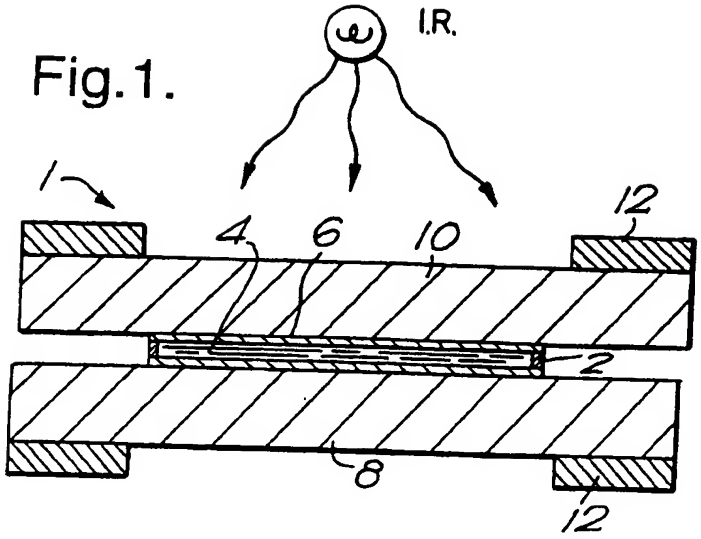


Fig.5.

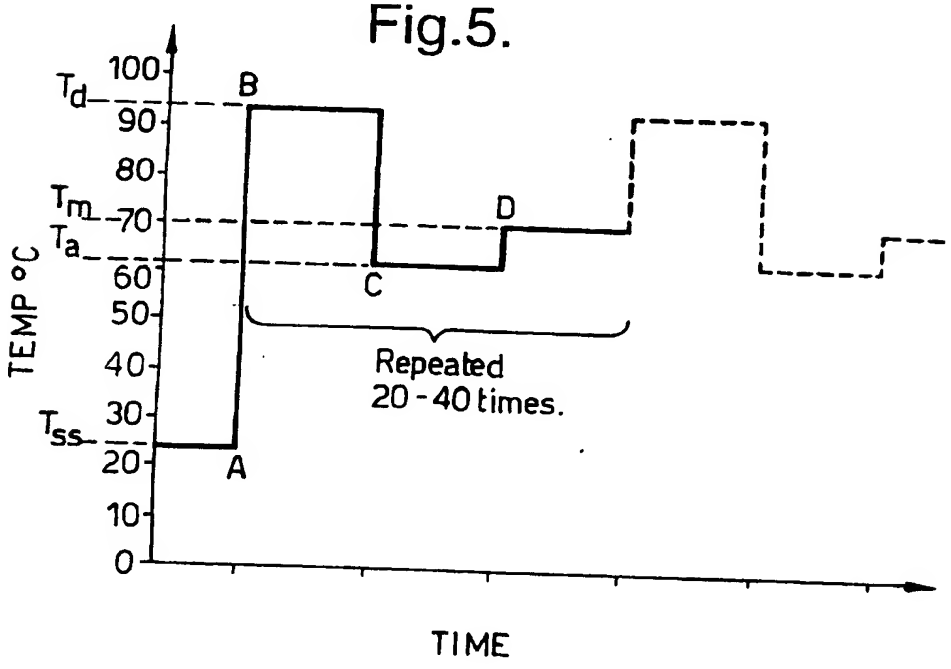


Fig.7.

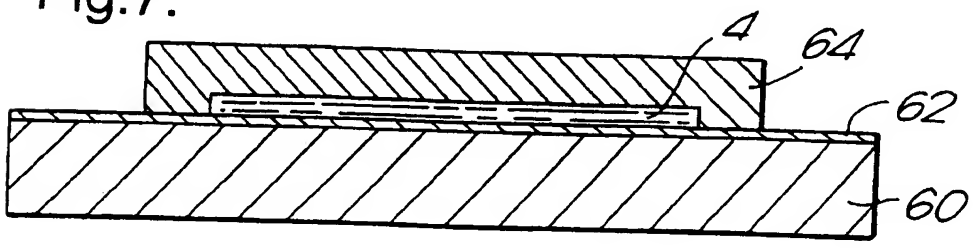


Fig.2.

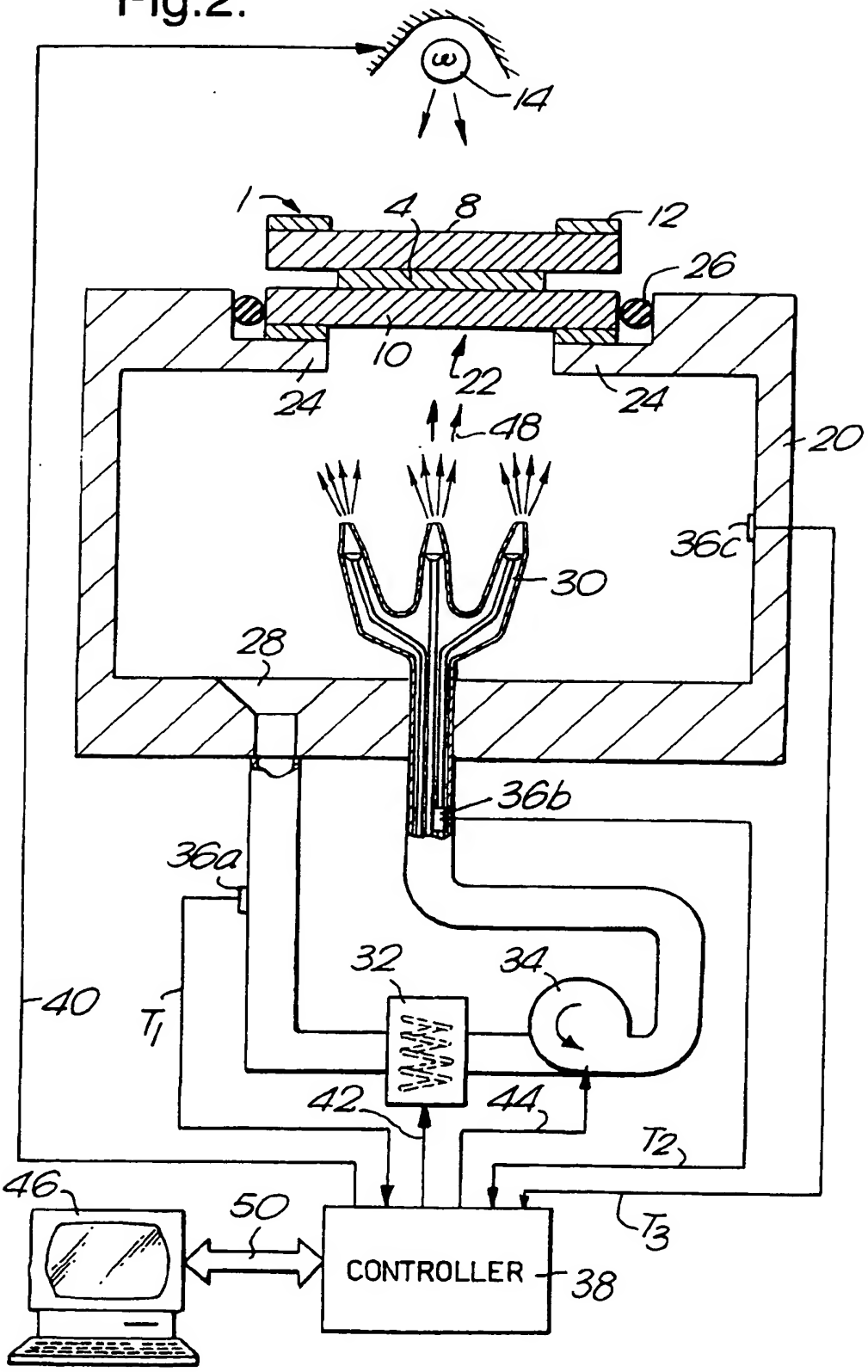

ω
14
1
4
8
12
26
10
22
24
24
48
20
36c
30
28
36b
36a
32
34
40
T1
42
44
T2
T3
46
50
CONTROLLER
38

Fig.3.

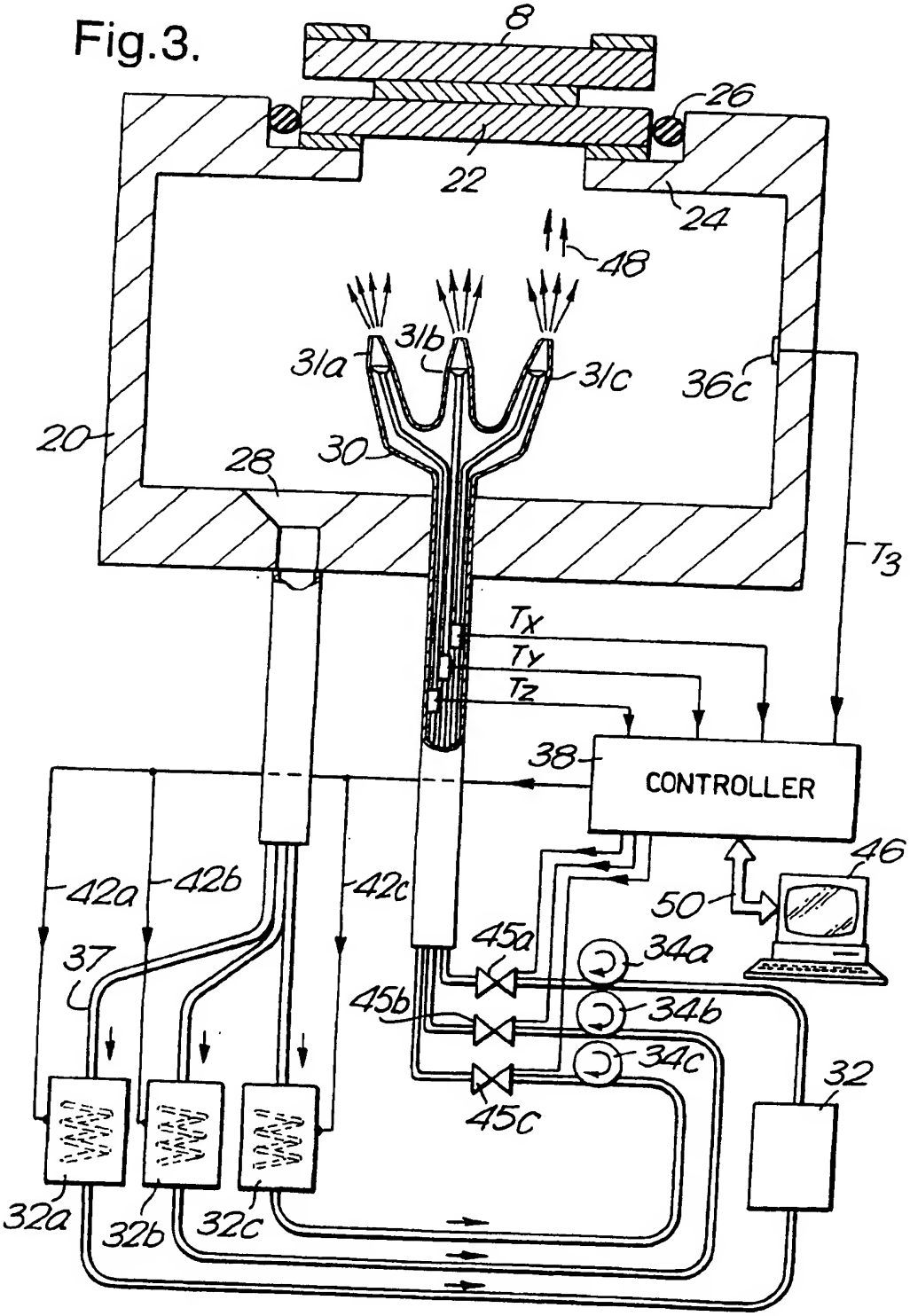

8
26
22
24
48
31a
31b
31c
36c
20
28
30
T3
Tx
Ty
Tz
38
CONTROLLER
46
50
42a
42b
42c
37
45a
34a
45b
34b
34c
32
45c
32a
32b
32c

Fig.4.

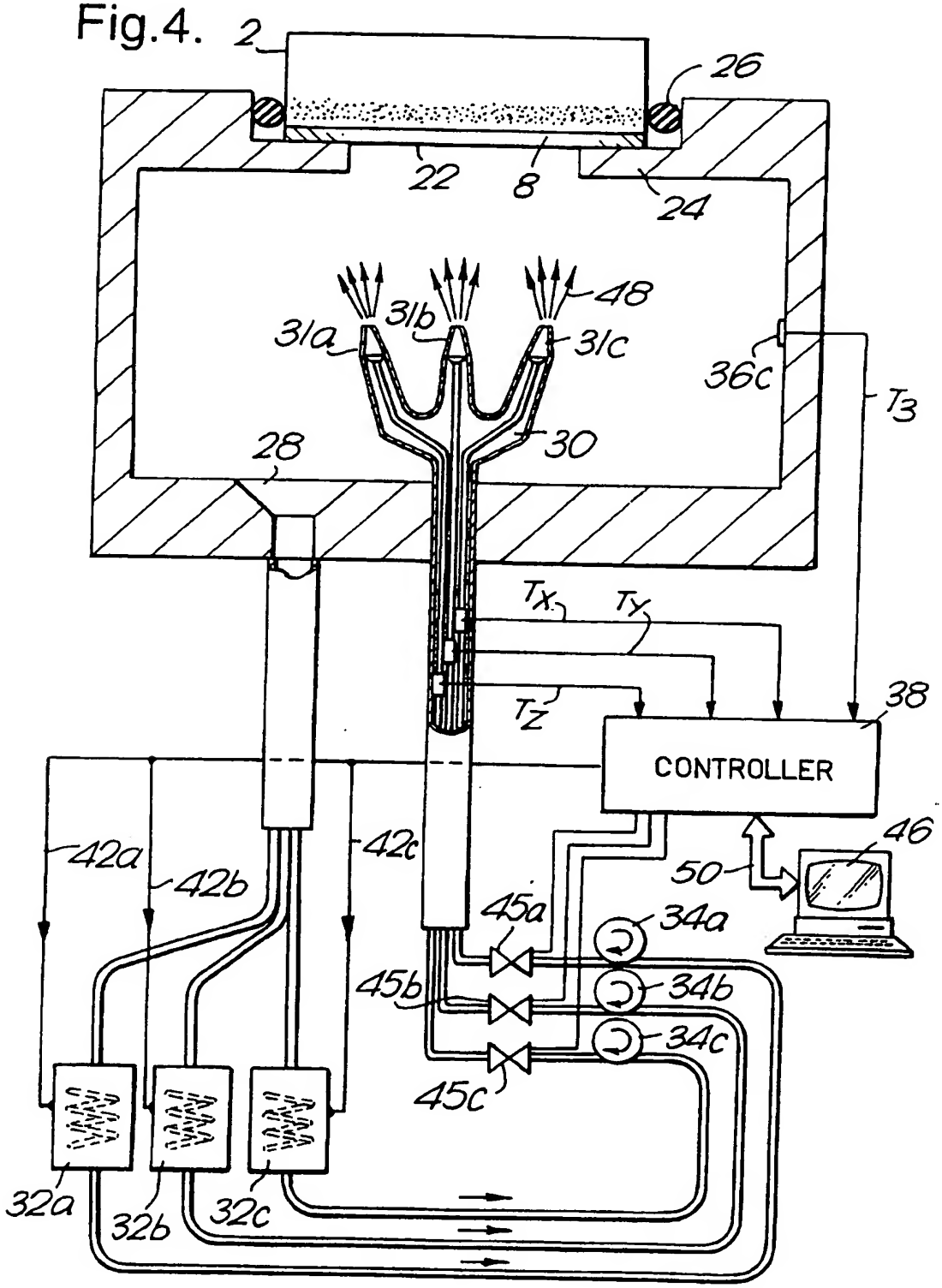
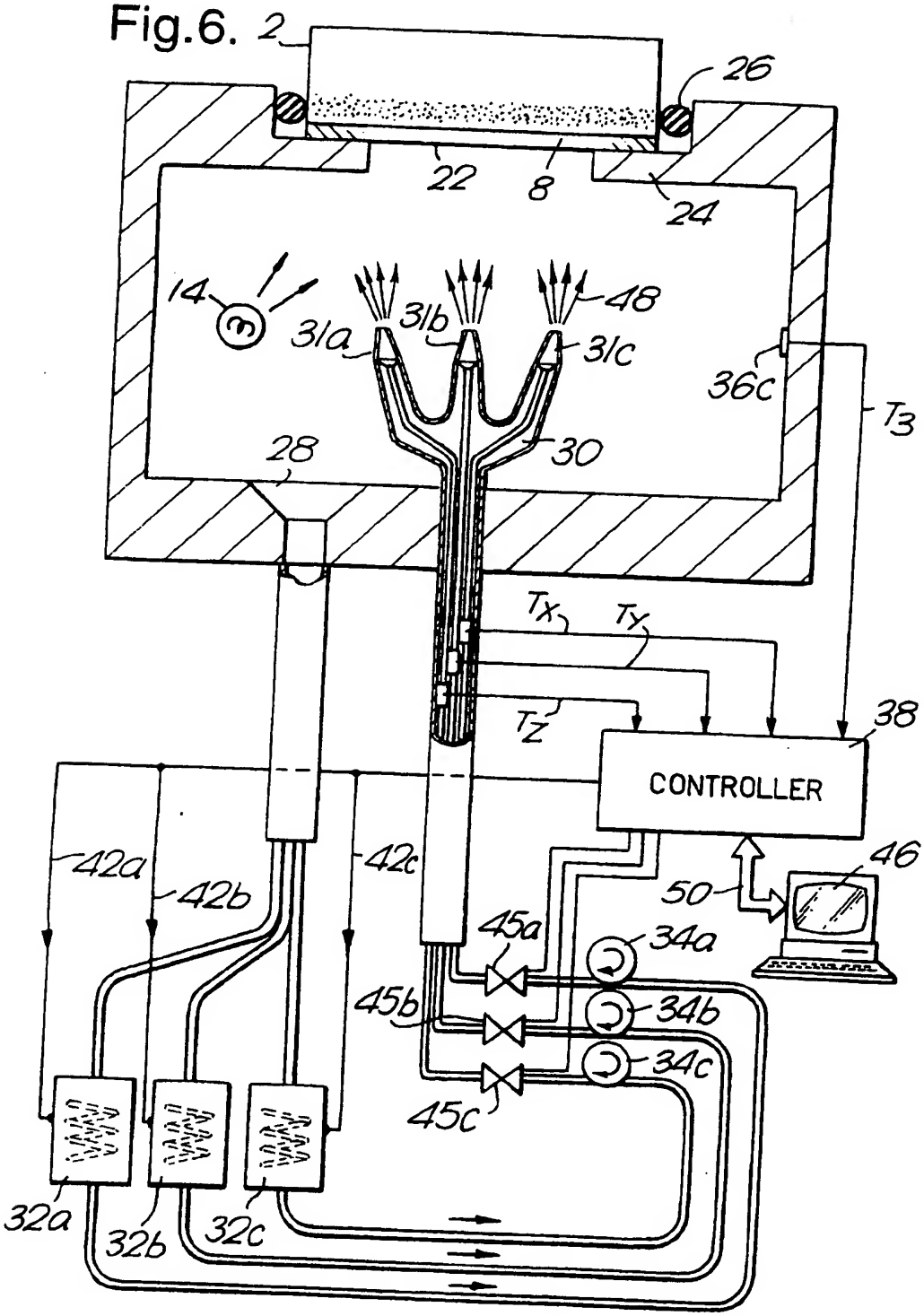

2
26
22
8
24
48
31a
31b
31c
36c
T3
28
30
Tx
Ty
Tz
38
CONTROLLER
46
50
42a
42b
42c
45a
34a
45b
34b
34c
45c
32a
32b
32c

Fig.6.


2
26
22
8
24
14
31a
31b
48
31c
36c
T3
28
30
Tx
Ty
Tz
38
CONTROLLER
46
50
42a
42b
42c
45a
34a
45b
34b
34c
45c
32a
32b
32c

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02421

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 B01L7/00 C12Q1/68 C12M1/38 B01J19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 B01L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 93 22058 A (UNIV PENNSYLVANIA) 11 November 1993 see page 18, paragraph 2	1,17,20, 21
X	see page 18, last line - page 19, line 4	14,15
X	see page 19, paragraph 2	14,15,34
Y	see page 19, last paragraph - page 20, paragraph 1	8,9
X	see page 20, paragraph 2 - page 21, paragraph 1	5,22-25
X	see page 21, paragraph 2	3,4,6
Y	see page 23, line 8 - line 11	11,26,27
X	see page 26, paragraph 1 ---	7
Y	EP 0 379 437 A (BERTIN & CIE) 25 July 1990 see column 5, line 14 - line 41 see column 6, line 22 - column 7, line 18; claim 6 --- -/--	8,9

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

2

Date of the actual completion of the international search	Date of mailing of the international search report
15 January 1998	23/01/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hocquet, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02421

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y A	EP 0 347 579 A (MESSERSCHMITT BOELKOW BLOHM) 27 December 1989 see column 11, line 28 - line 32 see column 11, line 56 - column 13, line 4; claim 15; figures 15-18	11,26,27 9
A	DE 195 19 015 C (INST PHYSIKALISCHE HOCHTECHNOL ;BIOMETRA BIOMEDIZINISCHE ANALY (DE) 5 September 1996 see column 2, line 40 - line 58 see column 1, line 67 - column 2, line 57 see column 3, line 19 - line 23; figure 1 see column 4, line 23 - line 44; figure 3	1
A	EP 0 542 422 A (GEN ATOMICS) 19 May 1993 see column 3, line 19 - line 40 see column 5, line 33 - column 6, line 20; figures see column 8, line 53 - column 9, line 14 see column 10, line 4 - line 48	1
A	US 5 455 175 A (WITTWER CARL T ET AL) 3 October 1995 see column 10, line 31 - column 11, line 1 see column 14, line 14 - line 65 see column 1, line 63 - column 2, line 17 see column 12, line 55 - line 63	1,17-21, 30
P,X	WO 96 41864 A (UNIV CALIFORNIA) 27 December 1996 see page 6, line 9 - page 7, line 35	1,26-29, 31
P,X	US 5 589 136 A (NORTHRUP M ALLEN ET AL) 31 December 1996 see column 1, line 25 - line 31 see column 5, line 9 - line 37 see column 7, line 12 - line 35; figure 3	1,3-7, 10-15
X	see column 7, line 36 - column 8, line 42; figures 4,6 see column 10, line 57 - line 65	22-27
X	see column 12, line 45 - column 13, line 19; figures 12-14	14,15
X	DE 38 08 942 A (BIO MED GMBH GES FUER BIOTECHN) 28 September 1989 see column 5, line 49 - column 7, line 60	2
X	WO 95 18676 A (ABBOTT LAB) 13 July 1995 see page 3, line 17 - page 4, line 13 see page 8, line 4 - page 10, line 26 see page 16, line 3 - line 6	2

-/--

2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02421

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 504 007 A (HAYNES JOHN L) 2 April 1996 see column 5, line 45 - line 48; figures 1-3 see column 7, line 8 - line 50 see column 8, line 51 - column 9, line 52; figure 7	2
X	US 5 508 197 A (HANSEN ANTHONY D A ET AL) 16 April 1996 see column 2, line 17 - line 48 see column 5, line 20 - line 56 see column 8, line 15 - line 25	2
A	EP 0 402 995 A (EASTMAN KODAK CO) 19 December 1990 see column 4, line 12 - line 30; figures 1,2 see column 4, line 55 - column 5, line 53; figure 4 see column 7, line 27 - line 41; figure 6	1,2

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/02421

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9322058 A	11-11-93	US 5304487 A	19-04-94
		US 5296375 A	22-03-94
		AT 155711 T	15-08-97
		AT 140025 T	15-07-96
		AT 140880 T	15-08-96
		AU 677780 B	08-05-97
		AU 4222393 A	29-11-93
		AU 680195 B	24-07-97
		AU 4222593 A	29-11-93
		AU 677781 B	08-05-97
		AU 4222693 A	29-11-93
		AU 4222793 A	29-11-93
		AU 677197 B	17-04-97
		AU 4223593 A	29-11-93
		CA 2134474 A	11-11-93
		CA 2134475 A	11-11-93
		CA 2134476 A	11-11-93
		CA 2134478 A	11-11-93
		DE 69303483 D	08-08-96
		DE 69303483 T	06-02-97
		DE 69303898 D	05-09-96
		DE 69303898 T	20-02-97
		DE 69312483 D	04-09-97
		EP 0637996 A	15-02-95
		EP 0637997 A	15-02-95
		EP 0639223 A	22-02-95
		EP 0637998 A	15-02-95
		EP 0637999 A	15-02-95
		HK 16897 A	13-02-97
		JP 7506430 T	13-07-95
		JP 7506431 T	13-07-95
		JP 7506256 T	13-07-95
		JP 7506257 T	13-07-95
		JP 7506258 T	13-07-95
		WO 9322053 A	11-11-93
		WO 9322054 A	11-11-93
		WO 9322421 A	11-11-93
		WO 9322055 A	11-11-93
		US 5635358 A	03-06-97
		US 5427946 A	27-06-95

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/02421

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9322058 A		US 5498392 A	12-03-96
		US 5587128 A	24-12-96
		US 5486335 A	23-01-96
		US 5637469 A	10-06-97
EP 0379437 A	25-07-90	FR 2642156 A	27-07-90
		AU 4963190 A	13-08-90
		CA 2025465 A	21-07-90
		DE 69007305 D	21-04-94
		DE 69007305 T	29-09-94
		ES 2053128 T	16-07-94
		WO 9008298 A	26-07-90
		JP 3503445 T	01-08-91
		US 5161609 A	10-11-92
EP 0347579 A	27-12-89	DE 3818614 A	07-12-89
		DE 3825907 A	01-02-90
		DE 58907327 D	05-05-94
		US 5252294 A	12-10-93
		DE 8817007 U	02-10-91
DE 19519015 C	05-09-96	WO 9637303 A	28-11-96
		EP 0772494 A	14-05-97
EP 0542422 A	19-05-93	NONE	
US 5455175 A	03-10-95	NONE	
WO 9641864 A	27-12-96	NONE	
US 5589136 A	31-12-96	WO 9700726 A	09-01-97
DE 3808942 A	28-09-89	NONE	
WO 9518676 A	13-07-95	AU 1527095 A	01-08-95
		CA 2178644 A	13-07-95
		EP 0739241 A	30-10-96
		JP 9508224 T	19-08-97
		US 5576218 A	19-11-96

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/02421

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5504007 A	02-04-96	NONE	
US 5508197 A	16-04-96	NONE	
EP 0402995 A	19-12-90	US 5460780 A	24-10-95
		CA 1329698 A	24-05-94
		CA 2016981 A,C	12-12-90
		CA 2016982 A,C	12-12-90
		DE 69014423 D	12-01-95
		DE 69014423 T	07-09-95
		DE 69015006 D	26-01-95
		DE 69015006 T	03-08-95
		DE 69030122 D	10-04-97
		DE 69030122 T	10-07-97
		EP 0402994 A	19-12-90
		EP 0606961 A	20-07-94
		HK 134995 A	01-09-95
		JP 3019700 A	28-01-91
		JP 3089939 A	15-04-91
		SG 16695 A	18-08-95
		US 5089233 A	18-02-92